culture pushed only relatively short distances up the Playas, the Uzpanapa, and the Coatzacoalcos. Whatever the importance of its influences on other Mesoamerican patterns, the Olmec civilization seems to have been restricted to a relatively narrow strip of coast from about the Laguna del Carmen across the swampy lowlands and the rugged Tuxtla mountains to the mouth of the Papaloapan.

BIOCHEMISTRY.—*Investigations concerning the hatching factor of the golden nematode of potatoes*, Heterodera rostochiensis *Wollenweber*.[1] Louis M. Massey, Jr.,[2] and A. Leslie Neal, Department of Biochemistry and Nutrition, Cornell University. (Communicated by G. Steiner.)

The golden nematode of potatoes or potato eelworm (*Heterodera rostochiensis* Wollenweber) is a plant-parasitic nematode which is responsible for the condition of soil known as "potato sickness." The nematode attacks the roots of the plant causing stunting and a reduction of yield which may be as great as 70 percent. It ranks among the most difficult pests to control. As yet, there is no known method of eradicating this organism once it has become established in a soil.

The life history of the golden nematode is described in Filipjev and Schuurmans Stekhoven, 1941. In brief, subsequent to the organism's invasion of and growth within the roots, the female body is transformed into a small round cyst which may contain from about 10 to 400 eggs. The cysts remain in the soil in a dormant state until exposed to an unidentified substance excreted by the potato root, which serves to stimulate hatching.

The first investigator to study the nature of the stimulant was Triffitt (1930) who found it to be non-volatile and moderately heat resistant. Hurst, as reported by Calam, Raistrick and Todd (1949), prepared a concentrate of the factor from potato soil leachings by evaporation and ethanol extraction procedures and with it was able to induce nematode hatching at a dilution of 1/500,000. Hurst concluded that the factor was an amino acid. Todd and coworkers in a series of publications (Calam, Raistrick, and Todd, 1949; Calam, Todd & Waring, 1949; Marrian, Russell, Todd, and Waring, 1949; Russell, Todd, and Waring, 1949a, 1949b; Marrian, Russell, and Todd, 1949) report attempts to isolate the active material and to synthesize active compounds. These workers concluded that the factor "is an acid probably containing a lactone group" and ascribed the name eclepic acid to this substance. The "high acidity and probable lactonic nature" led them to determine the activity of a number of tetronic acids and related compounds. Among those tested, anhydrotetronic acid was found to possess slight activity.

Investigations dealing with the hatching factor have been undertaken in this laboratory for the purpose of concentrating and identifying the active substance.

EXPERIMENTAL

Collection of leachings.—Tomatoes have been shown to excrete a golden nematode hatching factor which appears to be similar to that excreted by the potato plant (Russell, Todd, and Waring, 1949a). Since it was more convenient to use the former plant our studies have dealt with concentrating the factor from tomato leachings. The collection of relatively small volumes of leaching was accomplished by placing quart size "Sealright" cardboard containers under four-inch pots in which tomato plants were growing and allowing the leachings to drip directly into the containers. The contents of each container were frozen as soon as possible after collection. Frozen leachings have been found to retain their activity after storage periods of at least one year.

[1] Supported in part by grants from the New York State Agriculture and Market Golden Nematode Control Funds (Department of Plant Pathology, Cornell University). A preliminary report was presented at the meeting of the American Society of Biological Chemists at Cleveland, April 1951 (Federation Proc. **10**: 222. 1951). The authors wish to express their appreciation to W. F. Mai and B. F. Lownsbery, Jr., for the cyst material and for their many helpful suggestions.

[2] Present address: Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Md.

Assay method.—A comparison of the number of larvae hatched when cysts are immersed in solutions containing varying concentrations of the factor has served as a basis for estimating the relative concentration of the active substance (Fenwick, 1949, 1951a, 1951b, 1952; Lownsbery, 1951). The method used in this study is a slight modification of that developed by Lownsbery (1951) for testing the viability of larvae contained in nematode cysts.

A crude preparation of cyst material was obtained from heavily infested soil by flotation methods. The product was then air dried at room temperature and stored in a 50 percent relative humidity atmosphere. This crude preparation contained in addition to chaff, seeds, etc., approximately 7,000 cysts per g.

Aqueous extracts of this crude cyst material possessed slight hatching activity which appeared to be associated with the chaff constituent. Therefore, the chaff was removed by rolling the crude cyst material down a glass tube of 3 cm inside diameter and approximately 1 m in length. The tube which was fitted with a glass baffle about 10 cm from one end was inclined approximately 10° from horizontal, baffle end uppermost. Approximately 1 g of the crude cyst material was introduced into the upper end of the tube and the cysts and other spherical objects rolled out of the chaff by slowly rotating the tube around its longitudinal axis. The resulting material contained approximately 15,000 cysts per g and was found to be free from chaff.

Twenty mg of the chaff-free cyst material containing approximately 250 cysts were placed in a petri dish, and 20 ml of the solution to be assayed were then added. After an incubation period of 14 days at 21°C., the number of cysts present and the larvae which hatched were counted with the aid of a low power microscope. Since the number of cysts per dish is variable and a few larvae hatch from cysts placed in water, a distilled water blank as well as a solution of the initial leachings were incorporated in each assay for comparison purposes. A single source of cysts was used for an individual assay. The results are expressed as the number of larvae hatched per 100 cysts.

Concentration of the hatching factor.—Leachings were lyophilized at approximately 70 microns pressure and the residue extracted five times with 400 ml portions of absolute ethanol each time. The combined ethanolic extracts were then concentrated to 25 ml under 25 mm pressure. A white crystalline precipitate which formed during the concentration was removed by filtration and found to possess no activity. Upon the addition of 5 volumes of peroxide-free diethyl ether to the ethanolic filtrate additional inactive impurities were precipitated. After removal of the precipitate, evaporation of the ethanol-ether solution to dryness under 25 mm pressure left a residue which was not completely soluble in water. Extraction of this residue with 10 ml of distilled water and subsequent lyophilization of the aqueous solution yielded a yellow amorphous substance which is referred to as "concentrate-A."

Paper chromatography.—A study of the distribution of the hatching factor on paper chromatograms was made. Five μl of a solution (192 μg of solids per μl) of concentrate-A was applied to each of several strips of Whatman No. 1 paper (1½ x 25 inches). The strips were developed at 25°C. immediately after the spots had dried. Both ascending and descending developments were tried using 80 percent aqueous phenol, 50 percent phenol in 10 percent aqueous ethanol, and n-butanol saturated with water. After development, the strips were dried at room temperature in forced air for a 24-hour period. Beginning at one-quarter inch below the point of application for ascending developments, or one-quarter inch above the point of application for descending developments, the strips were cut into 1 inch segments and numbered consecutively. Each segment was eluted with 40 ml of distilled water which was subsequently divided into two equal portions for duplicate assays.

For comparison purposes, a "crude concentrate" of the hatching factor was prepared according to the method of Calam, Raistrick, and Todd (1949). Two μl of a solution of this product containing 180 μg of solids was subjected to paper chromatography using 80 percent aqueous phenol as the solvent. The distribution of activity on paper chromatograms of a mixture of the concentrates obtained by the two above procedures was also determined.

Leaf and root tissue preparations.—One hundred leaf punches (1 cm diam.) taken at random from ten six-week old tomato plants were floated upon 250 ml of distilled water in suitable containers and illuminated for periods of one and two days. At the end of each of these periods 20 ml aliquots of the water from each dish were assayed for activity.

One gram samples (fresh weight) of leaf and

root tissues were homogenized in a Potter-Elvehjem homogenizer under the following conditions.

1. Homogenized in distilled water at room temperature.
2. Homogenized in absolute ethanol at room temperature.
3. Homogenized in absolute ethanol at 0°C.
4. Steam blanched for 2 min. subsequent to homogenization in absolute ethanol at room temperature.
5. Lyophilized subsequent to homogenization in absolute ethanol at room temperature.

After homogenization the samples were centrifuged and the supernatant solution decanted. Each sediment was thoroughly extracted with absolute ethanol, centrifuged again and the supernatant added to the first extract respectively. These solutions were then evaporated to dryness under 15 mm. pressure and the residue taken up in 25 ml of distilled water for assaying.

RESULTS AND DISCUSSION

The cysts used for any individual assay were from a composite sample obtained from a single source in order to eliminate the difference in the viability of larvae in cysts collected at different times. Generally, during the hatching season duplicate assays did not vary from the mean by more than 10 percent.

All preparations of concentrates obtained possessed activity equivalent to or greater than that of the original leachings when diluted the appropriate amount with distilled water. For example, using duplicate assays the following average number of larvae hatching per 100 cysts were obtained: Distilled water, 200; original leaching, 600; concentrate-A (concentrated 4×10^4 times) diluted 4×10^4 times with distilled water, 805. Since a reliable quantitative assay for the factor was not available at the time these experiments were conducted the extent to which the preparations could be diluted and still exhibit hatching activity was not determined.

It has been observed that caution must be exercised during the process of concentrating solutions of the factor because of its lability towards heat and alkali. These properties are in accordance with those reported for eclepic acid.

The hatching factor was further concentrated by the technique of paper chromatography. Aqueous phenol, 80 percent, was found to be a satisfactory solvent for developing the chromatograms. Ascending developments with this solvent gave considerably sharper separations than did descending developments. Fig. 1 shows the distribution of activity on a typical strip when concentrate-A was developed for a distance of 19¼ inches at 25°C. using the ascending technique with the above solvent. The highest activity was found in the seventeenth segment, corresponding to a R_F value of 0.84. The average R_F value of six determinations was 0.84 ± 0.066. Weight determinations of the dried eluates from the various segments indicated that most of the solids remained in segments 2 to 5, inclusive. For example, after applying 950 μg to a strip, less than 10 μg of active residue was found to be eluted from the seventeenth segment.

The activity on the dry chromatograms was found to be quite rapidly destroyed upon exposure to air at room temperature. Elution immediately following the 24-hour air-drying period resulted in very little if any loss of activity. How-

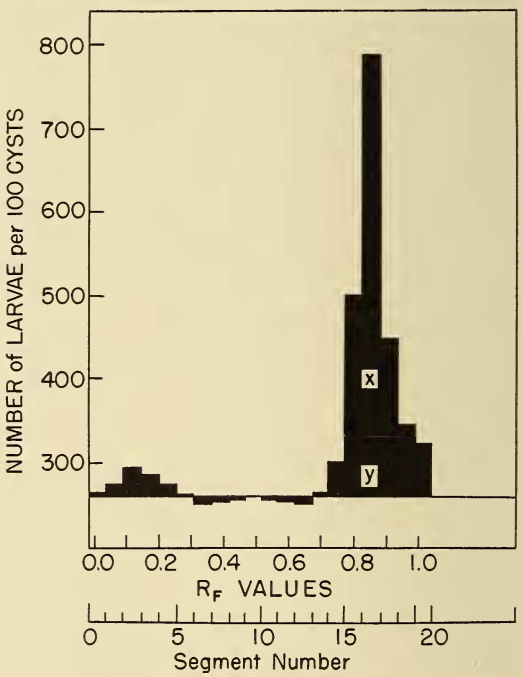

FIG. 1.—Distribution of activity on paper chromatogram of concentrate-A. A total of 950 μg of crude concentrate in 5.0 μl of water was applied to the paper. After development in 80 per cent phenol at 25° C. for a distance of 19¼ inches, the paper strips were dried and cut into one inch segments for elution and assaying. The values represented by X and Y indicate the activity of the most active segment after exposure to the laboratory atmosphere for 2 and 4 days, respectively, following the air-drying period.

ever, approximately 50 to 100 percent loss of activity from the most active segment occurred when the strips were permitted to remain exposed to the laboratory atmosphere for two and four days, respectively, after the drying period (see X and Y, Fig. 1).

The distribution of activity obtained when the crude concentrate prepared according to the method of Calam, Raistrick and Todd (1949), was chromatogrammed is shown in Fig. 2. The peak of highest activity corresponds to a R_F value of 0.83. When mixtures of this preparation and concentrate-A were chromatogrammed there was no change in the R_F value.

Concentration of the factor by continuous ether extraction of an aqueous sulfuric acid solution has been used by previous workers (Calam, Raistrick, and Todd, 1949). From the data presented in Table 1 it is apparent that the extracted material from concentrate-A possesses about the same activity as the non-extracted residue. However, the activity of more purified preparations obtained from paper chromatograms does not appear to be extracted with ether either from an aqueous sulfuric acid solution or directly from the paper chromatogram. The data presented in Table 2 are from a typical experiment in which the most active segments from four paper chromatograms were used. In this experiment 0.98 mg of concentrate-A was applied to each strip. In each treatment where ether was employed it was evaporated off after the extraction and the residue, if any, taken up in 40 ml of distilled water for duplicate assays. For continuous ether extraction the segments were eluted with 7.5 ml water and the solution acidified with 1.5 ml of 2N H_2SO_4. After the extraction period the aqueous phase was neutralized with NaOH and diluted to 40 ml for assaying. The inhibitory effect of Na_2SO_4 is shown by the lack of activity of solutions 2 and 3b (Table 2). This effect was not noted above due to the dilution employed. It is of interest to note the inactivity of the ether extracts (3a and 4a) and the high activity of the aqueous eluate (4b) from the strip which had previously been extracted with ether in a Soxlet apparatus for a period of 12 hours.

The presence of a hatching agent in tomato leaf tissue is indicated by the fact that water upon which leaf punches were floated was found to possess hatching activity. About twice the number of larvae were hatched in the presence of the water upon which illuminated leaf punches

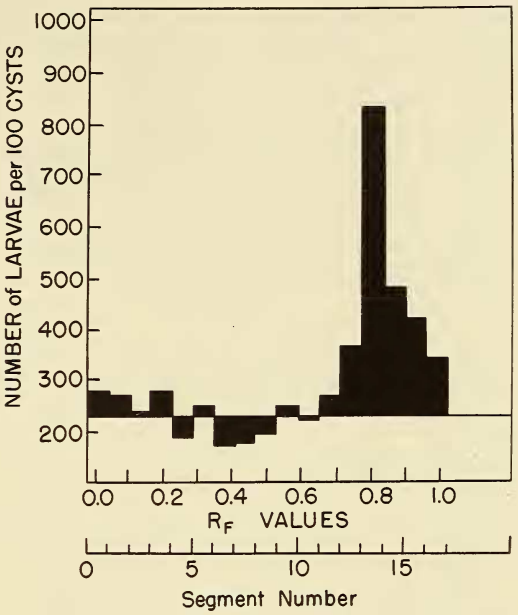

FIG. 2.—Distribution of activity on paper chromatogram of the product prepared by the procedure of Calam, Raistrick and Todd (1949). A total of 27 μg of crude product in 2.1 μl of water was applied to the paper. After development in 80 percent aqueous phenol at 25°C. for a distance of 16⅜ inches, the paper strips were dried and cut into one inch segments for elution and assaying.

were floated for a period of one day as was hatched by an equivalent volume of leachings (see Table 3). At the end of two days illumination there was about an eight fold difference in the number of larvae hatched.

The occurrence of a hatching stimulant in tomato leaves was further established by assaying extracts of the homogenized tissue. A comparison of the activity of leaf homogenates prepared under different conditions is presented in Table 4. The activity of root tissue homogenates prepared under the same conditions is also shown. Although the values for the number of larvae

TABLE 1.—ACTIVITY OF ETHER EXTRACTED MATERIAL FROM AQUEOUS SULFURIC ACID SOLUTION OF CONCENTRATE-A

Fraction	Weight	Concentration of solution assayed	Increase in number of larvae hatched above that of the blank
	grams	*μg/ml*	*per μg/ml of solids*
Ether phase	0.15	1.5	61
Aqueous phase	0.23*	3.5*	71

* Corrected for the Na_2SO_4 formed by neutralizing the H_2SO_4 previous to assaying.

hatched are quite low due to seasonal variations, there is a definite significant difference between several of the treatments and the distilled water blank. In the case of treatments 3, 4, and 5 (Table 4) the activity of the homogenates of both leaf and root tissue was equal to or greater than that of leachings. Steam blanching or lyophilizing the leaf tissue previous to homogenizing in absolute ethanol at room temperature appeared to be the most satisfactory treatment for this tissue. By far the most active root homogenates were obtained when the roots were homogenized in absolute ethanol at 0°C. Homogenates of the tissues in distilled water at room temperature showed no activity in the case of leaf tissue and a slight, if significant, activity in the case of root tissue.

TABLE 2.—ETHER SOLUBILITY OF CONCENTRATES OBTAINED FROM PAPER CHROMATOGRAMS

Treatment of most active segment	Larvae per 100 cysts*
1. Eluted with 7.5 ml distilled water	273
2. Eluted with 7.5 ml distilled water, 1.5 ml of 2N H_2SO_4 added and solution immediately neutralized with NaOH	12
3. Eluted and acidified as in 2, then continuously extracted with ether for a period of 12 hours:	
a. Ether phase	26
b. Neutralized aqueous phase	10
4. Extracted in Soxlet apparatus for a period of 12 hours:	
a. Ether	7
b. Water eluate of segment after ether extraction.	244
Distilled water blank	24

* Average of duplicate assays.

TABLE 3.—ACTIVITY OF WATER UPON WHICH ILLUMINATED TOMATO LEAF PUNCHES WERE FLOATED

Assay solution	Larvae per 100 cysts*
Distilled water	1.5
Tomato leachings	16
Water upon which leaf punches were floated:	
1 day illumination	30
2 days illumination	130

* Average of duplicate assays.

TABLE 4.—ACTIVITY OF TOMATO TISSUE HOMOGENATES

Treatment*	Larvae per 100 cysts†	
	Leaf tissue	Root tissue
1. Homogenized in distilled water at room temperature	1.5	5.5
2. Homogenized in absolute ethanol at room temperature	5	8
3. Homogenized in absolute ethanol at 0°C.	11	35
4. Steam blanched, homogenized in absolute ethanol at room temperature	16	19.5
5. Lyophilized, homogenized in absolute ethanol at room temperature.	16.5	11

Tomato leachings; 11 larvae per 100 cysts.†
Distilled water; 2 larvae per 100 cysts.†
* One gram (fresh weight) of tissue used in each preparation.
† Average of duplicate determinations.

SUMMARY

A method for obtaining a concentrate of the golden nematode hatching factor has been described.

By employing the technique of paper chromatography the factor was found to have a R_F value of 0.84 when 80 percent aqueous phenol was used as the solvent.

In a fairly pure state the factor was found to be ether insoluble.

A hatching agent has been shown to be present in leaf tissue as well as in root tissue of the tomato plant.

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BOTANY.—*Realignments in the Bromeliaceae subfamily Tillandsioideae.* LYMAN B. SMITH, Department of Botany, U. S. National Museum, and COLIN S. PITTENDRIGH,[1] Department of Biology, Princeton University.

It has long been evident that no author has been consistent or logical in delimiting the genera of the subfamily Tillandsioideae of the Bromeliaceae. Virtually all useful phylogenetic characters are limited to the petals, stamens, and pistil, yet available material is so frequently inadequate in these parts, that there is a tremendous temptation to base genera on other characters. The assumption has been that certain habital characters are correlated with floral ones. This is true in a single instance, the absence of spines on the leaves of the Tillandsioideae. All other correlations in the subfamily are incomplete to begin with as in the case of the distichous arrangement of flowers that partially characterizes *Tillandsia* and *Vriesia*, or else they have broken down with the discovery of additional species.

We do not believe in making changes on well established systems such as the latest monograph of the family (Mez in Engler, Das Pflanzenreich IV. **32**) unless something demonstrably better can be offered, but the three genera noted below, *Thecophyllum* André, *Cipuropsis* Ule, and *Chirripoa* Suesseng., are now useless even in an artificial system. Although the generic position of many species must remain in doubt until good flowers are obtained, we are transferring all species on the basis of such evidence as is available. We preface our treatment of *Thecophyllum* and *Cipuropsis* by a concept of *Vriesia* Lindley emended appropriately to include these entities in the sense used by Mez in his last monograph. *Guzmania* requires no emendation to accommodate *Thecophyllum* in the original sense of André.

Vriesia Lindl. emend. Smith & Pittendrigh

Inflorescentia simplex vel paniculata, ea paniculata cum bracteis primariis vel parvis et inconspicuis vel conspicuis et ramos plus minusve obtegentibus; sepalis liberis; petalis vel in tubum brevem sepalis valde superatum connatis vel omnino liberis, appendiculatis; ovario supero vel paulo infero.

Lindley's type species, *V. psittacina*, is gamopetalous, but this fact has been overlooked and the genus characterized as polypetalous, as will be detailed in another paper. As defined above, *Vriesia* contains all the species of the Tillandsioideae with a primary type of gamopetaly, that is, with petals truly fused or connate and not merely agglutinated and more or less interlocking as in the secondary type that characterizes *Guzmania* and *Mezobromelia*. Since it also contains polypetalous species, its basic character remains its appendaged petals.

Thecophyllum André

(Structure of corolla noted where known)

Theccophyllum André, Bromel. Andr. 107. 1889 = **Guzmania** R. & P. Fl. **3**: 37. 1802, in all probability. Of the two original species, the first, *T. wittmackii*, is undoubtedly a *Guzmania*, while the second, *T. poortmanii*, very likely is also although its corolla is still unknown to us. See below.

Thecophyllum André emend. Mez, Bull. Herb. Boiss. II. **3**: 131. 1903 = **Vriesia** Lindl. Bot. Reg. **29**: *pl. 10*. 1843.

T. acuminatum L. B. Smith, Contr. Gray Herb. **117**: 30, *pl. 2, figs. 28, 29*. 1937 = **Vriesia attenuata** Sm. & Pitt. nom. nov. Not *Vriesia acuminata* Mez & Wercklé, Bull. Herb. Boiss. II. **4**: 868. 1904. Petals appendaged—LBS.

[1] This author acknowledges assistance in the course of his work from the Eugene Higgins Memorial Fund, Princeton University.